

# Accelerated perineural invasion in colitis-associated cancer

## A retrospective cohort study

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### Abstract

Perineural invasion (PNI) is a prognostic factor in patients with colorectal cancer. Neurotrophic factors, molecular determinants of PNI, are altered in their expression levels in patients with ulcerative colitis. In this study, we evaluated the frequency of PNI in colitis-associated cancer (CAC) and sporadic cancer.

We retrospectively reviewed 778 colorectal cancers with pathological T3-T4 in 761 patients all of whom were surgically resected without preoperative treatment. The lesions were classified into either CAC or sporadic cancer based on the clinical information. Clinicopathological findings including PNI were compared between CACs and sporadic cancers. Moreover, we analyzed the risk factors for positive PNI by multivariate analysis using a logistic regression model.

Ten of the cancers (1.3%) were diagnosed as CACs, and the remaining 768 as sporadic cancers. CACs were characterized by being nonobstructive and predominantly located in the rectum. The CACs had a larger size and more frequent undifferentiated histology than sporadic cancers. PNI was observed more frequently in CACs (90%) than in sporadic cancers without obstruction (45%,  $P = .007$ ). On multivariate analysis, CAC was one of the significant factors associated with PNI (odds ratio: 9.05,  $P = .040$ ).

Our results suggest that CAC was more likely to exhibit PNI than sporadic colorectal cancer.

**Abbreviations:** APC = adenomatous polyposis coli, BDNF = brain-derived neurotrophic factor, CA = carbohydrate antigen, CAC = colitis-associated cancer, CEA = carcinoembryonic antigen, CRC = colorectal cancer, GCNF = glial cell-derived neurotrophic factor, IBD = inflammatory bowel disease, NGF = nerve growth factor, PNI = perineural invasion, RFS = recurrence-free survival, RUNX = Runt-related transcription factor, Trk = tropomyosin receptor kinase, UC = ulcerative colitis.

**Keywords:** colitis-associated cancer, perineural invasion, sporadic colorectal cancer

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## 1. Introduction

In addition to lymphatic and vascular invasion, malignant cells can invade nervous structures and spread along nerve sheaths as a metastatic route.<sup>[1]</sup> In several malignancies such as head and neck and prostate cancers, it has been well documented that perineural invasion (PNI) plays a critical role in tumor growth and progression by mutual signaling interactions among tumors, nerves, and stroma.<sup>[2–4]</sup> In colorectal cancer (CRC), it was recently revealed that PNI is associated with local and distant organ recurrence and a worse postoperative survival rate.<sup>[5–10]</sup>

As clinical factors, the placement of a metallic colon stent as a bridge to surgery and obstruction has been shown to potentially increase the frequency of PNI in colon cancers, as reported by our group and others.<sup>[11–13]</sup> In addition, several neurotrophic factors such as nerve growth factor (NGF), glial cell-derived neurotrophic factor (GCNF), and brain-derived neurotrophic factor (BDNF), have been identified as determinants of PNI in rodent tumor models as well as various human neoplasms.<sup>[14]</sup> These molecules have been suggested to be involved in the crosstalk between tumor cells and nerves.<sup>[14]</sup>

Ulcerative colitis (UC) is one of the prevalent inflammatory bowel disease (IBD) entities, and CRC is a well-recognized complication especially when patients have risk factors such as a longstanding extensive disease, a young age at diagnosis, and persistent inflammation.<sup>[15–18]</sup> There is accumulating evidence

suggesting that chronic intestinal inflammation is accompanied by alterations of the enteric nervous system. Neurotrophins such as NGF, GDNF, BDGF, and neurotrophin-3 as well as their receptors are highly expressed in the intestine of human IBD patients and several animal models of IBD,<sup>[19,20]</sup> whereas regulatory roles for several neurotrophins are recognized in experimental inflammation of the gut.<sup>[21]</sup> We considered that the frequency of PNI in colitis-associated cancer (CAC) may be different from that in sporadic CRC due to altered expressions in neurotrophins and their receptors.

In the present study, we investigated the frequency of PNI in CACs and sporadic cancers and addressed the association of CACs with PNI.

## 2. Methods

Using our database, we searched for consecutive patients with pathological T3-T4 primary colorectal cancer who underwent tumor resection in the Department of Surgical Oncology, University of Tokyo Hospital between July 2013 and January 2019. Cancers arising in IBD patients were included whereas patients with anal fistula-associated cancer were excluded from the study. Patients who underwent surgery for perforation, or received preoperative chemotherapy and/or radiotherapy, stent placement for malignant obstruction were also excluded.

We retrieved demographic data and tumor-related parameters including age, sex, body mass index, preoperative levels of tumor markers, that is, carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9, tumor location, and obstruction from medical charts. Histopathological diagnoses of the primary tumor using such factors as tumor type (sporadic cancer or CAC), size, histological grade, depth, lymphatic and venous invasion, and PNI were made by experienced gastrointestinal pathologists according to the eighth edition of the American Joint Committee

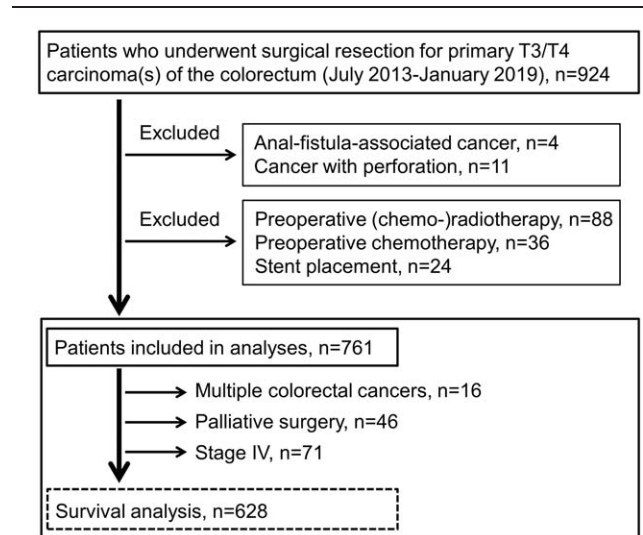


Figure 1. Study flow graph.

on Cancer Staging System.<sup>[22]</sup> In our hospital, the upper normal limits of serum CEA and CA 19-9 are 5.0 ng/mL and 37U/mL, respectively. Recurrence-free survival (RFS) was defined as time between surgery and first recurrence in curatively operated CRC patients without distant metastases.

Comparisons of parameters among groups were performed using the Wilcoxon rank-sum, Fisher exact, and Chi-square tests, applying Yates' correction as appropriate. Predictive factors for PNI were estimated by univariate and multivariate analyses using a logistic regression model. RFS curves were drawn using the Kaplan–Meier method and differences were evaluated by the log-rank test. Statistical analysis was performed using JMP

Table 1

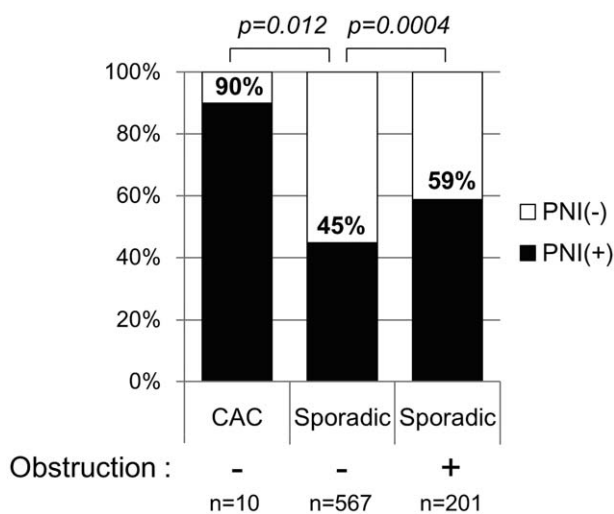
Comparison of patient and tumor characteristics between colitis-associated and sporadic colorectal cancers.

Variable		UC patients with CAC (n=10)	Patients with sporadic CRC (n=751)	P-value
Age	Mean ± SD	49.6 ± 10.8	67.3 ± 12.1	<.0001
Sex	Male	9 (90%)	414 (55%)	.049
	Female	1 (10%)	337 (45%)	
BMI (kg/m <sup>2</sup> )	Mean ± SD	22.3 ± 2.3	22.7 ± 3.8	.73
CEA	Elevated	4 (40%)	392 (52%)	.65
CA 19-9	Elevated	5 (50%)	180 (24%)	.12

Variable		CAC (n=10)	Sporadic CRC (n=768)	P-value
Primary site	Colon	2 (20%)	508 (66%)	.004
	Rectum	8 (80%)	260 (34%)	
Size of tumor, mm	Mean ± SD	66 ± 38	49 ± 20	.008
Obstruction	No	10 (100%)	567 (74%)	.072
	Yes	0 (0%)	201 (26%)	
Histology	Differentiated	6 (60%)	695 (90%)	.011
	Others	4 (40%)	73 (10%)	
Depth	pT3	9 (90%)	508 (66%)	.080
	pT4	1 (10%)	260 (34%)	
Lymphatic invasion	Present	7 (70%)	305 (40%)	.055
Venous invasion	Present	10 (100%)	607 (79%)	.23
PNI	Present	9 (90%)	371 (48%)	.010

BMI=body mass index, CA 19-9=carbohydrate antigen 19-9, CAC=colitis-associated cancer, CEA=carcinoembryonic antigen, CRC=colorectal cancer, Differentiated=well or moderately differentiated adenocarcinoma, PNI=perineural invasion, SD=standard deviation.



**Figure 2.** Frequency of perineural invasion in pathological T3/T4 colorectal cancers according to colitis and obstruction. CAC=colitis-associated cancer, PNI=perineural invasion.

14.2.0 (SAS Institute Inc, Cary, NC). *P*-values of <.05 were considered significant.

This study was conducted following approval by the ethics committee of the University of Tokyo Hospital (No. 3252-(8)).

### 3. Results

During the study period, 924 patients underwent surgery for pathological T3-T4 carcinoma of the colorectum. As shown in Figure 1, 761 patients were enrolled in the present study (Fig. 1). Among 778 CRCs in these patients, 10 cancers (1.3%) were CACs that had developed in 10 UC patients. Two sporadic CRCs were simultaneously resected in 15 patients and 3 in 1 patient. First, the clinicopathological findings were compared between CAC and sporadic CRC. As shown in Table 1, UC patients were younger than patients with sporadic CRC, and predominantly male. CACs were more frequently found in the rectum than sporadic cancers (80% vs 34%, *P* = .004). In addition, tumor size was larger in CACs by 17mm (*P* = .008). Despite this finding, obstructive cancers were found only in sporadic cases (26%, *P* = .072). Histological analysis showed that cancer types other than differentiated adenocarcinoma were more often found in CACs than in sporadic CRCs (40% vs 10%, *P* = .011). PNI was more frequently observed in CACs (90%) than in sporadic

cancers (48%, *P* = .010). There was no significant difference in lymphatic and venous invasion, or pT stage distribution.

The frequency of PNI in CACs and sporadic CRCs was further analyzed according to obstruction and type of CRC. As shown in Figure 2, the positive rate of PNI (90%) was notably higher in CACs with pT3/T4 than in nonobstructive sporadic pT3/T4 tumors (45%, *P* = .007). In sporadic CRCs, PNI was observed in 59% of obstructive cancers, a significantly higher rate than the nonobstructive cancers (*P* = .0006).

We attempted to identify the factors associated with PNI in CRCs. Univariate analysis revealed that CAC, rectal cancer, differentiated histology, pT4, lymphatic and venous invasions were potential factors related to PNI, which were further entered into a multivariate model. As summarized in Table 2, all the above factors were independently associated with PNI; CAC showed an odds ratio of 9.05 for PNI against sporadic cancer (*P* = .040).

Finally, RFS curves were compared in patients with and without PNI. Here we analyzed 628 patients who underwent curative-intent surgery for a pathological T3/T4 CRC without distant metastases, as shown in Figure 1. The median follow-up period was 36.5 months. The RFS of patients with PNI-negative cancer was significantly better than that of patients with PNI-positive cancer (3-year rate: 84% vs 65%, *P* < .0001; Fig. 3).

### 4. Discussion

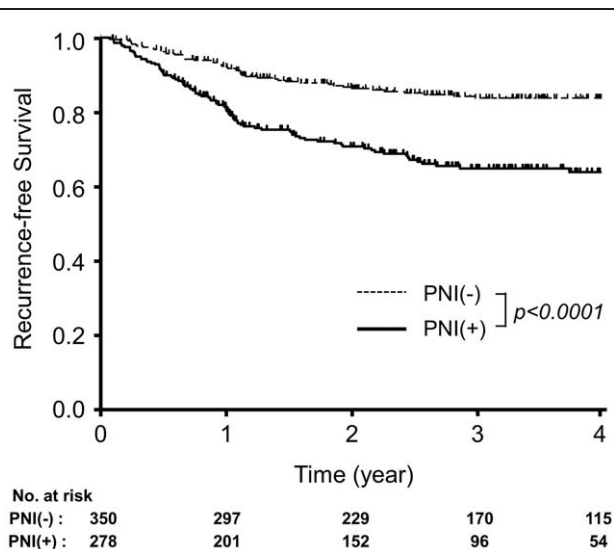
In previous studies, tumor depth, regional lymph node metastases, and lymphatic and venous invasions were found to be factors associated with PNI<sup>[5,10,23,24]</sup>; some of their associations were also confirmed in our study. The relationship between histological grades and PNI remains controversial.<sup>[5,8,10,12]</sup> Neoadjuvant treatments such as chemotherapy and radiotherapy were reported to reduce PNI in rectal cancer.<sup>[10]</sup> Recently, we demonstrated obstruction as an independent factor of positive PNI.<sup>[13]</sup> In the present study, we demonstrated that CACs carry a high-risk for PNI independently of the aforementioned risk factors.

Not only prominent histological features such as frequent undifferentiated and invasive histology,<sup>[25,26]</sup> but also specific genetic alterations characterize CACs. Sporadic CRCs develop by genetic alterations in the *adenomatous polyposis coli*, *RAS*, and *p53*, in the adenoma-carcinoma sequence, whereas alterations in other genes are also implicated in the dysplasia-carcinoma sequences in CACs.<sup>[25,27]</sup> We previously found that DNA copy numbers and in situ expression of runt-related transcription factor (RUNX)3 in the rectal mucosa of CAC patients were lower

**Table 2**  
Univariate and multivariate analysis of factors associated with perineural invasion.

Variable		Univariate	Multivariate	
		<i>P</i> -value	OR (95% CI)	<i>P</i> -value
CRC type	CAC (vs sporadic)	.005	9.05 (1.10–74.35)	.040
Primary site	Rectum vs (colon)	.011	1.42 (1.03–1.97)	.035
Size of tumor, mm	≥50 (vs <50)	.29		
Obstruction	Present (vs absent)	.0001	1.49 (1.03–2.16)	.034
Histology	Differentiated (vs others)	.013	2.38 (1.36–4.15)	.002
Depth	pT4 (vs pT3)	<.0001	1.91 (1.35–2.69)	.0002
Lymphatic invasion	Present (vs absent)	<.0001	3.15 (2.28–4.36)	<.0001
Venous invasion	Present (vs absent)	<.0001	2.27 (1.51–3.42)	<.0001

CAC=colitis-associated cancer, CI=confidence interval, CRC=colorectal cancer, Differentiated=well or moderately differentiated adenocarcinoma, OR=odds ratio.



**Figure 3.** RFS curves of T3/T4 colorectal cancer patients without distant metastases who underwent curative-intent surgery stratified by PNI. The bold line indicates the RFS curve of patients with PNI-positive CRC, and the dashed line that of patients with PNI-negative CRC. CRC=colorectal cancer, PNI=perineural invasion, RFS=recurrence-free survival.

than those in UC patients without neoplasms.<sup>[28,29]</sup> RUNX3 downregulates the expression of tropomyosin receptor kinase B, a high-affinity receptor for BDNF and neurotrophin-4, and controls the neurotrophin receptor phenotype *in vivo*.<sup>[30]</sup> Based on our results, we speculate that the interaction of neurotrophins and their receptors may be enhanced by reduced RUNX3 expression, which subsequently contributes to the accelerated PNI in CACs. Further studies are required to address the possible underlying mechanism.

Nationwide population-based cohort studies in 2 different countries reported that CAC patients exhibited an increased mortality.<sup>[26,31]</sup> A meta-analysis showed poorer overall survival in CAC patients than in patients with sporadic CRC.<sup>[32]</sup> On the other hand, several groups including us demonstrated that PNI was correlated with increased local recurrence as well as decreased RFS and overall survival in CRC.<sup>[10,13]</sup> Based on these findings, there is a possibility that the poor survival rate can be attributable to innately frequent PNI in CACs.

We previously demonstrated that obstruction was significantly associated with a higher rate of PNI in sporadic T3/T4 colon cancer.<sup>[13]</sup> In the present study, PNI was analyzed in sporadic cancers of both the colon and rectum as counterparts of CACs. The same findings were confirmed in pathological T3-T4 CRCs by multivariate analysis in the present study.

Our study contains several limitations. In our series, the expressions of neurotrophins and their receptors were not compared between non-neoplastic mucosa and neoplasia in IBD patients. Second, we cannot exclude the possibility that sporadic-type cancers developed in the IBD patients; however, pathologists confirmed that all of the 10 CACs had the pathological and immunohistochemical features of cancers arising from a colitis background such as p53 overexpression. Moreover, the small number of CACs made it difficult to perform survival analysis to address the prognostic significance of PNI in the UC patient group alone. Lastly, we did not encounter CACs arising in patients with the Crohn disease.

In conclusion, we demonstrated that CAC was an independent factor associated with PNI in pathological T3 and T4 CRC. The molecular mechanisms responsible for the relationship between colitis-associated carcinogenesis and PNI need to be clarified.

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